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Microalgae *for* Biofuel Production *and* CO2 Sequestration

Bei Wang
Christopher Lan

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MICROALGAE FOR BIOFUEL PRODUCTION AND CO_2 SEQUESTRATION

CHRISTOPHER Q. LAN
AND
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PREFACE

Owing to their vast diversity and high growth rate, microalgae offer numerous advantages as the most promising photosynthetic organisms for biofuel production and CO_2 bio-sequestration. Two different processes have been proposed for these purposes: microalgal farming and ocean fertilization. This book focuses primarily on the former while providing a brief introduction to the latter.

Chapter 1 briefly discusses the diversity of microalgae, the concept of photosynthesis, and the microalgal species that have been most studied for biofuel production and CO_2 sequestration in microalgal farming settings;

Chapter 2 discusses the nutritional and environmental requirements of microalgae and the media and cultivation systems commonly used for microalgal farming;

Chapter 3 discusses the use of microalgae for CO_2 sequestration using two different approaches: microalgal farming and ocean fertilization;

Chapter 4 introduces a variety of biofuels to be produced using microalgae as feedstock or cell factories;

Chapter 5, which is co-authored by Courchesne N.M. Dorval, Albert Parisien, Bei Wang and Christopher Q. Lan, discusses the principles and recent developments in the metabolic channelling for enhancing production of lipids, the feedstock for biodiesel production;

Chapter 6 presents a novel approach involving factor grouping and Box-Behnken experimental design for microalgal medium optimization;

Chapter 7, which is co-authored by Yanqun Li, Bei Wang, Nan Wu and Christopher Q. Lan, presents the experimental evidence demonstrating the potential of the green alga *Neochloris oleoabundans* for lipid production.

CONTENTS

Chapter 1

MICROALGAE FOR CO_2 SEQUESTION AND BIOFUEL PRODUCTION

1.1. MICROALGAE

Microalgae, defined conventionally for the purpose of this book as all unicellular and simple multi-cellular photosynthetic microorganisms including both prokaryotic microalgae (cyanobacteria) and eukaryotic microalgae, are the most important primary producer of the oceans. They are also widely found in other habitats such as lakes, rivers, ponds, wet lands, deserts and even the north and south poles. It was estimated that there are one to ten million microalgal species on the earth (Bold, 1985) and more than 40,000 species have been identified to date. The vast diversity of microalgal species and their capability of high-efficiency photosynthesis for fast growing, solar energy capturing and CO_2 fixation make them the most promising bio-species for CO_2 bio-sequestration and biofuel production.

The microalgal species that have been identified to date can been classified into 11 divisions (10 eukaryotic microalgae plus the prokaryotic cyanobacteria) according to their photosynthetic pigment composition, biochemical constituents, ultrastructure, and life cycle as listed in Table 1.1. Among these microalgae, six classes of them are of primary importance to biofuel production: diatoms (*alias Bacillariophyceae*, belonging to the division Chrysophyta), green algae (Class Chlorophyceae), golden-brown algae (Class Chrysophyceae), prymnesiophytes (Class Prymnesiophyceae), eustigmatophytes (Class Eustigmatophyceae), and blue-green algae or cyanobacteria (Class Cyanophyceae) (Sheehan et al., 1998).

Table 1.1. Main pigments, storage products, and cell coverings of different divisions of microalgae (Barsanti, 2006)

Division	Pigments					
		Chlorophylls	Phycobilins	Carotenoids	Xanthophylls	Storage Products
Cyanophyta	blue-green algae	A	c-Phycoerythrin c-Phycocyanin Allophycocyanin Phycoerythrocyanin	β-Carotene	Myxoxanthin Zeaxanthin	Cyanophycin (argine and asparagine polymer) Cyanophycean starch (α-1,4-glucan)
Prochlorophyta	blue-green algae	a,b	Absent	β-Carotene	Zeaxanthin	Cyanophycean starch (α-1,4-glucan)
Glaucophyta	green algae	A	c-Phycocyanin Allophycocyanin	β-Carotene	Zeaxanthin	starch (α-1,4-glucan)
Rhodophyta	Red Algae	A	r,b-Phycoerythrin r-Phycocyanin Allophycocyanin	α- and β-Carotene	Lutein	Floridean starch (α-1,4-glucan)
Cryptophyta	blue- green algae	a,c	Phycoerythrin -545 r-Phycocyanin	α-,β-, and ε-Carotene	Alloxanthin	starch (α-1,4-glucan)
Heterokontophyta	Golden or Brown algae	a,c	Absent	α-,β-, and ε-Carotene	Fucoxanthin Violaxanthin	Chrysolaminaran (β-1,3-glucan)
Haptophyta	green algae	a,c	Absent	α- and β-Carotene	Fucoxanthin	Chrysolaminaran (β-1,3-glucan)
Dinophyta	dinoflagellates	a,b,c	Absent	β-Carotene	Peridinin, Fucoxanthin, Diadinoxanthin Dinoxanthin Gyroxanthin	starch (α-1,4-glucan)
Euglenophyta	flagellate	a,b	Absent	β- and γ-Carotene	Diadinoxanthin	Paramylon (β-1,3-glucan)
Chlorarachniophyta	mainly green algae	a,b	Absent	Absent	Lutein Neoxanthin Violaxanthin	Paramylon (β-1,3-glucan)
Chlorophyta	green algae	a,b	Absent	α-,β-, and γ-Carotene	Lutein Prasinoxanthin	starch (α-1,4-glucan)

Diatoms

Diatoms, also called Bacillariophyceae, are a class belonging to division Chrysophyta. The cells of diatoms are golden-brown because of the presence of high level of fucoxanthin, a photosynthetic accessory pigment. Several other xanthophylls are present at lower levels, as well as β-carotene, chlorophyll α and chlorophyll c. The main storage compounds of diatoms are triglycerides (TAGs) and chrysolaminarin, a β-1,3-linked carbohydrate. Diatom cell wall contains substantial quantities of polymerized Si. This unique feature has important implications for media preparation and costs in a commercial production facility, because silicate is a relatively expensive chemical. On the other hand, deficiency of silicate can promote lipid (TAG) accumulation in diatoms. It can be employed to provide a controllable means to induce lipid synthesis in a two-stage production process. Diatoms are the most common and widely distributed groups of microalgae on earth. They dominate the phytoplankton of the oceans and are also commonly found in fresh- and brackish waters.

Green Algae

Green algae, including divisions Prochlorophyta and Chlorophyta, have chlorophyll a and chlorophyll b as photosynthetic pigments. Green algae are believed to be the evolutionary progenitors of higher plants and have received more attention than other groups of algae. *Chlamydomonas reinhardtii* (and closely related species), a member of this group, has been studied extensively. It was the first alga to be genetically transformed. Another genus of green algae that has been studied extensively is Chlorella. Several green algae, for instance, *Neochloris oleoabundans*, are known to be able to accumulate large quantities of lipids and efficient in CO_2 fixation (Li et al. 2008a; Liet al. 2008b), making them attractive candidates for combined CO_2 fixation and biofuel production.

Golden-Brown Algae

Golden-Brown algae include the chrysophytes and the synurophytes. They are similar to diatoms with respect to pigment composition. Some chrysophytes have lightly silicified cell walls. They are found primarily in freshwater habitats. Lipids and chrysolaminarin are the most common carbon storage materials of this group.

Prymnesiophytes

Prymnesiophytes (haptophytes) are primarily marine organisms and account for a substantial proportion of the primary productivity of tropical oceans. Some prymnesiophytes produce algal blooms, which may cause serious problems. Prymnesiophytes are often of a golden-brown color because of the presence of the yellow-brown accessory pigments, diadinoxanthin and fucoxanthin. Lipids and chrysolaminarin are the major storage form of this group of algae.

Eustigmatophytes

This group represents an important component of the "picoplankton", which is comprised of a group of small microalgae with cell size in the range of 2-4μm in diameter. The genus Nannochloropsis is one of the few marine species in this class and is commonly found in the world's oceans. Chlorophyll a is the only chlorophyll present in Eustigmatophyte cells. They contain however several xanthophylls that serve as accessory photosynthetic pigments.

Blue Green Algae (Cyanobacteria)

As mentioned previously, Cyanobacteria are not microalgae but a group of photosynthetic bacteria. They are treated here as microalgae in a broader sense for convenience. Cyanobacteria are prokaryotes that contain no nucleus, no chloroplasts, and have a different gene structure than all other microalgae. There are approximately 2,000 species of cyanobacteria, which have been found in a diversity of different habitats. Some members of this group can assimilate atmospheric N_2 and therefore eliminate the need to provide fixed nitrogen for cell growth. A few commercial facilities have been built for cultivation of cyanobacteria, (e.g., *Spirulina platensis*) for production of health foods and other novel products. No member of this class is known to produce significant quantities of storage lipids.

1.2. PHOTOSYNTHESIS

Photosynthesis is the process photosynthetic species, including microalgae, use to capture light energy to produce glucose and other organic carbons from

CO_2. Light energy is converted in the photosynthetic process to chemical bonding energy stored in cell materials (biomass). Photosynthesis involves two major reaction sequences: the light-dependent reactions (the light reactions) and the light-independent reactions (the dark reactions). In the light reactions, light energy is captured and converted to energy currency, NADPH and ATP. The dark reaction involves a sequence of reactions that fix and reduce inorganic carbon utilizing the ATP and NADPH generated in the light reaction. As shown in equation 1.1, the overall result of photosynthesis is that carbon is converted from CO_2 to carbohydrates, $[CH_2O]_n$, using light energy. The carbohydrates are subsequently converted to other cell materials for cell growth and cell maintenance.

$$n\,CO_2 + nH_2O + \text{light} \xrightarrow[\text{Chlorophyll } a]{} (CH_2O)n + nO_2 \qquad (1.1)$$

1.2.1. The Light Reaction

The light reaction, or more precisely the light-dependent reaction, is the first stage of photosynthesis. In this process light energy is converted to chemical energy in the form of energy-carriers ATP and NADPH. NADPH is also one of the major carriers of the reducing power that is required for the reductive anabolism of cells. There are two types of photosynthesis according to the donors of electrons: oxygenic photosynthesis and anoxygenic photosynthesis. In oxygenic photosynthesis, the electron donor is water, producing molecular oxygen as a by-product. In anoxygenic photosynthesis, various electron donors such as H_2S might be used.

The major mechinary required for light reactions include phototosystem I (PS I), Photosystem II (PS II), the photosynthetic electron tranfer chain (ETC) and the ATP synthase. Photosystems I and II are protein complexes containing light capturing pigments, which are responsible for absorbing light energy. Different pigments contained in cells are important characteristics in the taxonomy of microalgae. As shown in Figure 1.1, the light-dependent reactions begin in PS II, when a particular chlorophyll molecule of PS II absorbs a photon to activate an electron (i.e., the electron attains a higher energy level). Because the high energy state of an electron is very unstable, the electron is then transferred through the ETC. As a result, the electron flows from PS II to PS I, where the electron gets the energy from another photon. In the end, the electron is transferred to $NADP^+$, which serves as the final electron acceptor for the ETC and is reduced to NADPH. As will be discussed more in detail later, one major component of the photosynthetic ETC is cytochrome b6f, which couples with ATP synthase in a process called photophosphorylation to generate ATP molecules.

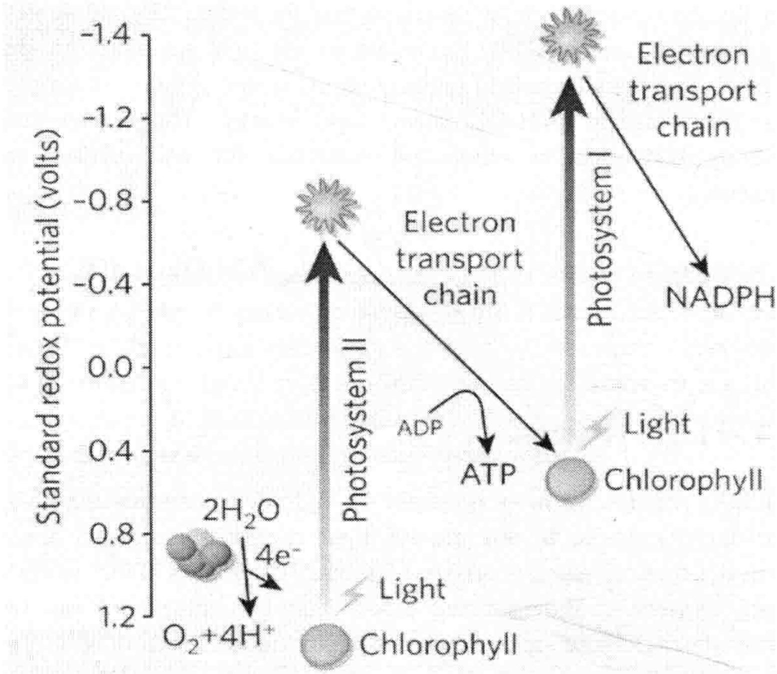

Figure 1.1. The light-dependant reactions of photosysynthesis (Allen & Martin, 2007)

In eukaryotic photosynthetic species such as plants and microalgae, photosynthesis takes place in a specialized organalle called chloroplast. As shown in Figure 1.2, chloroplasts are flat discs usually of 2 to 10 micrometers in diameter and 1 micrometer thick. The chloroplast consists of an inner membrane and an outer membrane, which are separated by the intermembrane space. The material inside chloroplast is called stroma. Chloroplast contains one or more molecules of small circular DNA and some ribosomes. However, most of its proteins are encoded by genes contained in the host cell nucleus and manufactured by ribosomes in the cytosol, with the proteins transported to the chloroplast. Within the stroma are stacks of thylakoids, which are called grana. A thylakoid has a flattened disk shape. Inside a thylakoid is an empty area called the thylakoid space or lumen. While the thylakoid membrane houses all the machinery for the light-dependant reactions and therefore is the location the light reaction takes place, the dark reaction takes place in the stroma of the chloroplast.

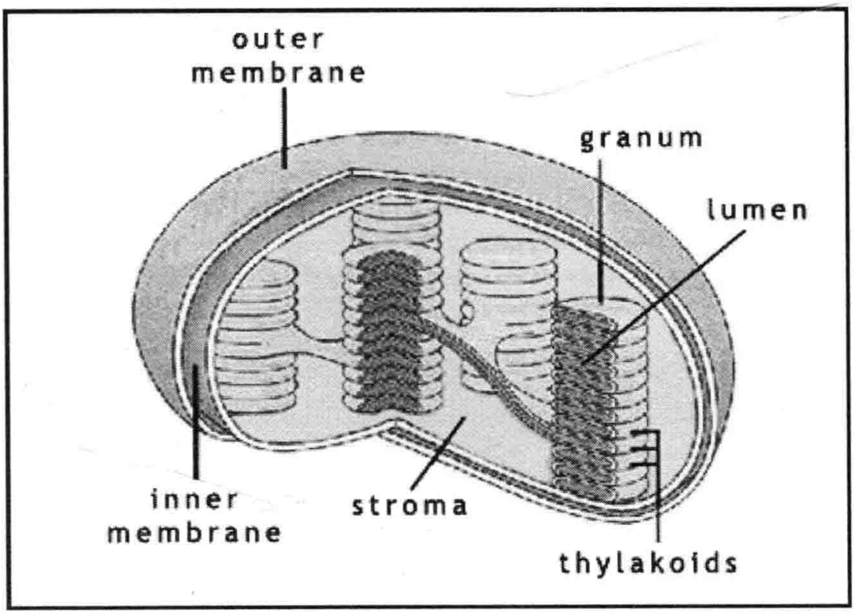

Figure 1.2. *The schematic diagram of the structure of a chloroplast*. Thylakoids are a phospholipid bilayer membrane-bound compartment. A granum is a stack of thylakoids folded on top of one another. The stroma is the fluid space within the chloroplast. The lumen is the fluid filled space within a thylakoid (image source: http://www.helpsavetheclimate.com/photosynthesis.html).

A more detailed decription of the light-dependant reaction machinary is shown in Figure 1.3, which includes the PS I, the photosynthetic ETC (including plastoquinone (PQ), cytochrome b6f, and plastocyanin (PC)), PS II, and the ATP synthase. In photophosphorylation, the activated electron is first accepted by PQ, the primary electron acceptor. Then, the cytochrome b6f uses the energy of electron to pump protons from the outside of the thylakoid membrane (the chloroplast stroma) to the inside of the thylakoid (the thylakoid lumen) to create a proton gradient across the thylakoid membrane. The proton gradient drives the ATP synthase, which locates across the thylakoid membrane, to form ATP. Photophosphorylation may occur in two different ways: noncyclic photophosphorylation and cyclic photophosphorylation. In non-cyclic photophosphorylation, cytochrome b6f uses the energy of electrons from PSII only and the electron passed to PS I, which is re-activated by the photon absorbed